Research Article

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Phenolic content and antimicrobial activities of date palm (*Phoenix dactylifera L.*) fruits and leaves

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ABSTRACT

This study aimed to investigate total phenolic content and antibacterial activities of different extracts recovered from date palm fruit and leaves using various solvents, including methanol, ethanol, and water. The results showed that the highest phenolic content was found in leaf methanol extract, followed by leaf ethanol extract, and then followed by seed methanol extract (74.4, 67.3, and 64.7 mg/g, respectively). Whereas the date methanol extract had the lowest phenolic content (5 mg/g). The study was also scrutinized to find the antibacterial inhibitory property in the leaves and fruits of date palm against four bacterial species (*Escherichia coli, Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*) using the well-diffusion method and minimum inhibitory concentration (MIC). Date leaves' extracts showed inhibitory effect on the growth of *S. aureus* and *B. subtilis* and resistance to *P. aeruginosa* and *E. coli*. MIC of methanol and ethanol (70%) leaves' extract was 100 and 250 mg/ml for *S. aureus* and *B. subtilis,* respectively; however, all the bacteria have resistance to date fruits' extracts.

KEY WORDS: Antimicrobial activity, date palm, minimum inhibitory concentration, phenolic content

INTRODUCTION

The date palm (*Phoenix dactylifera* L.), a tropical and subtropical tree, belonging to the family Palmae (*Arecaceae*) is one of the humankind's oldest cultivated plants. Date palm is the most successful and commercially important crop in the hot-arid regions of the world, for example, Saudi Arabia, Emirates, and Egypt. In these countries, date palm products are commonly used for human and animal consumption, pharmaceuticals, cosmetics, carpentry, and firewood. A large number of date palm cultivars are known; however, until now, only a few of these cultivars have been evaluated for chemical composition and nutritional quality (Chao and Krueger, 2007). Date palm fruits are a good source of vitamins minerals simple carbohydrate and dietary fibers (El-Sohaimy and Hafez, 2010). Pulps of dates hold easily digestible sugars (70%), mostly glucose, sucrose, and fructose, dietary fibers, and enclose less proteins and fats (Al Farsi and Lee, 2008). Moreover, date fruits possess antioxidant and antimutagenic properties (Vayalil, 2002), attributable to their high levels of polyphenolic compounds and vitamins (Mansouri, *et al.*, 2005; Al-Turki, *et al.*, 2010). The date fruit pulp is rich in phytochemicals such as phenolics, sterols, carotenoids, anthocyanins, procyanins, and flavonoids. The ratio and concentrations of these constituents depend on the type of the fruit, stage of the fruit picking, location, and soil conditions; these phytochemicals also contribute to the nutritional and organoleptic properties of the fruits (Abdul and Alliath, 2008). There was also found a good correlation between the total phenolic content (TPC) and antioxidant activities of the nonvolatile extracts (Faqir *et al.*, 2012). Considering the nutritional importance of dates, studying their biochemical composition and nutritional quality is increasingly being recognized as a worthy and important task. The aim of the present study is to analyze the phenolic profile of date palm fruits and leaves and to evaluate their functional properties such as antioxidant and antimicrobial activities to confirm their nutritional benefits.

MATERIALS AND METHODS

Date fruits were collected from a date farm in Abu Dhabi, UAE, and brought to the laboratory in sterile containers. Date leaves were collected from a random cultivar in Al Ain city. The used solvents of methanol, ethanol, and ethyl acetate were obtained also from Sigma Company.

Preparation of Date Palm Tree Fruit and Leaves Extract

Date fruits were opened, and the seeds were removed. The seedless fruits and leaves were washed with distilled water and kept in the hot oven at 60°C for 1 week to dry. Then, the dried fruits and leaves were grinded separately in a blender to make a powder. 10 g of the grinded date fruits were measured and added to methanol in a conical flask and left in the shaker incubator 150 rpm (rotation/min) for 2 days. On the other hand, 21 g of the grinded leaves were measured and added to three different solvents, 7 g each, which are methanol, and ethanol in conical flasks and left in the shaker incubator 150 rpm (rotation/min) for 2 days. The mixture were filtered, poured into a Petri plate, and kept in the oven to dry for 1 week, respectively. The same solvent (methanol) was used to transfer the date extract from the Petri plate to a test tube. The extract was covered with aluminum foil (to avoid oxidation) and stored at the room temperature for the future analysis.

TPC using Spectrophotometer

TPC of the date palm fruits was determined in extracts using Folin-Ciocalteu assay (El Sohaimy, 2013). To three different concentrations, 0.1, 0.3, and 0.5 ml of crude extract, distilled water was added, respectively, to make it 1 ml and tested for phenolic content separately.

1 ml of extract was transferred to test tube containing 1 ml of Folin-Ciocalteu reagent followed by addition of 10 ml sodium carbonate (Na_2CO_3) (7 g/100 ml the contents were incubated for 5 min at 50°C; then, the absorbance was measured at 550 nm against different concentrations of gallic acid as standard. The phenolic content was expressed as mg gallic acid equivalents per gram of extract (dry weight sample).

Thin Layer Chromatography (TLC)

TLC was used to separate the extracts compounds to their components. The gallic acid solution was used as a standard. Components of the samples were compared with the standard color using the ultraviolet light hand lamp was adjusted to 302 nm.

Antimicrobial Activity

The antibacterial activity of the date palm fruit and leave extracts was tested using the agar well-diffusion method against four bacterial species *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis,* and *Staphylococcus aureus*, which obtained from Accuvis Bio Laboratory, Abu Dhabi University. Holes were punched out from the nutrient agar and the following volumes were added using a micropipette: 75, 100, and 150 µl. The concentration of the date fruit sample was kept 400 mg/ml and that of leaf extract was 250 mg/ml. Solvent in which extracts were suspended was used as a negative control; antibiotic amoxicillin was used as a positive control. Then, the diameter of the inhibitory zone for each hole was measured in millimeter using a transparent ruler. The results were analyzed in duplicates and the means values are presented.

Minimum Inhibitory Concentration (MIC)

The MIC of an antimicrobial agent is the lowest (i.e., minimal) concentration of the antimicrobial agent that inhibits a bacterial isolate from multiplying and producing visible growth. Extracts were prepared in different concentration 100, 250, 400 mg/ml, and added into a sterile tube containing 1 ml of nutrient broth and bacterial cultures were inoculated as 105 cells/ml and incubated at 37°C for 18-24 h. Controls were incubated like nutrient broth with only extract and another tube with only bacterial cultures. After incubation, results were examined for turbidity in the tubes with control and also to confirm the results, loop of culture was inoculated in agar plates by streaking.

RESULTS

TPC using Spectrophotometer

The results in Table 1 showed that both fruit and leaf have phenolic content. The concentration of phenolic content was calculated by the standard curve of gallic acid (Figure 1). Results revealed that the highest phenolic content was found in leaf methanol sample (74.4 mg/g), followed by leaf ethanol sample (67.3 mg/g), and followed by seed methanol sample (64.7 mg/g). On the other hand, the methanol date sample had the lowest phenolic content (5 mg/g). However, it was observed ethanol and ethyl acetate of date fruit does not showed any result.

TLC

The result in Figure 2 showed that the closest colors to the standard gallic acid (sample 7) are ethanol leaf (sample 1), methanol leaf (sample 3), and methanol date (sample 5), which indicates that the highest phenolic content exists in the leaf methanol extract, then the leaf ethanol extract, followed by the date methanol extract. However, further studies are required to identify the different types of phenolic compounds exist in the date palm fruits and leaves. This can be done by gas chromatography-mass spectrometry technique.

Table 1: Phenolic content of date and leaf extracts

Sample	Concentration (ml)	Quantity (g)	Extract volume (ml)	Phenol content μg/ml	Multiplied by 1000 (μg)	Divided by 1000 (mg)	Phenol content (mg)
Date methanol	0.1	10	10	50.210	50210	50	5
Leaf methanol	0.1	7	10	521.338	521338	521	74.4
Leaf ethanol	0.1	7	10	470.860	470860	471	67.3

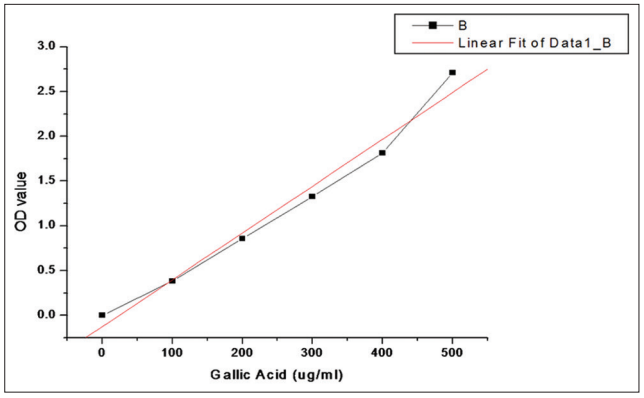

Figure 1: Standard curve of gallic acid

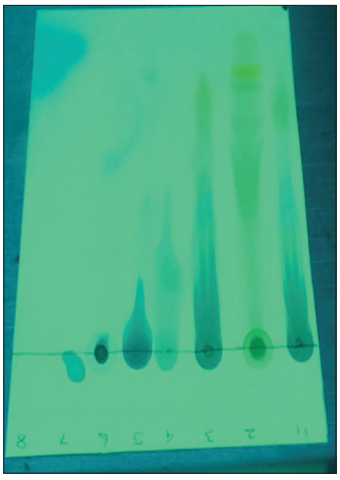

Figure 2: Thin-layer chromatography of date and leaves extracts

Antimicrobial Activity

The antibacterial activity of date palm fruits extracts of two different solvents (70% ethanol and methanol) against four bacteria study revealed that date fruit extracts are not capable of inhibiting the growth of Gram-positive and Gram-negative bacteria that are used in this study (Table 2). On the other hand showed the antibacterial activity of date palm leaves extracts isolated by solvents (70% ethanol and methanol) against four bacterial species *E. coli*, *P. aeruginosa*, *B. subtilis,* and *S. aureus* (Table 2). Antibiotic amoxicillin was used as positive control for all bacterial strains.

The methanol extract had shown high inhibitory zone against *B. subtilis* and *S. aureus* compared to 70% ethanol (Figure 3). When the added volume of extract increases, the inhibitory zone increases as well. The highest inhibition zone (15 mm) was observed against *S. aureus* by the leaf methanol extract.

Among the four bacteria, *S. aureus* is more sensitive to the leaves extract as it showed the highest inhibitory zone, then *B. subtilis* came next. On the other hand, both *E. coli* and *P. aeruginosa* showed resistance against all the leaves extract as no inhibitory zone appeared around the holes in the agar plates (Figures 1 and 2). This means that the Gram-positive bacteria are more sensitive to the leaves extracts than the Gram-negative bacteria.

When compared with the antibiotic (positive control) zone of inhibition, the inhibition zones by the leaf extracts were comparatively less except the methanol leaf extract against the *S. aureus*. The difference between the methanol leaf extract and the antibiotic was 2 mm; 15 mm by methanol 150 μl, 17 mm by amoxicillin 50 μl against *S. aureus*. The difference between the positive control and *B. subtilis* was recorded very high.

The antibacterial activity of date and leaves extracts with different solvents (ethanol and methanol) against four strains of bacteria showed that the methanol leaf extract had the highest inhibitory zone against the different bacterial strains, while the date fruits have not shown any inhibitory zone against the different bacterial strains. Because of the high content of carbohydrates found in the date fruits, the phenolic compounds might be wrapped by them. These results approved the strong relationship between the phenolic content and the antibacterial activity.

MIC

The MIC was conducted to identify the lowest concentration that extract will inhibit the growth of bacteria. This method was done on three bacteria which are *B. subtilis*, *E. coli*, and *S. aureus* against three different extract concentration which are 100, 250, and 400 mg/ml of ethanol and methanol extract. *S. aureus* showed inhibitory effect for the leaves extracts and at all the different concentrations and its MIC in all the three extract was 100 mg/ml. *B. subtilis* showed inhibited effect at 250 and 400 mg/ml of two different

leaves extracts: Methanol and 70% ethanol. The MIC for *B. subtilis* was 250 mg/ml of ethanol extract and methanol extract. *E. coli* and *P. aeruginosa* were strong enough to resist the effect of the leaves extracts concentrations even at high concentration that did not show any inhibitory effect (Table 3).

DISCUSSION

Our results are in agreement, to some extent, with that reported by Al-Farsi *et al*., 2005 who found that total phenol contents of dates were in methanol and water extracts, respectively. The present study also proved that the most phenolic content was found in methanol extract.

Date palm leaf extracts showed good inhibitory effect against Gram-positive bacteria. Moreover, our methanol extracts' inhibitory results were in line with the studies of Jaroszynska, 2003 who found that the greatest extraction is done by methanol. The inhibition zone seen by methanol leaf extract ranges from 12 to 15 mm against *S. aureus*

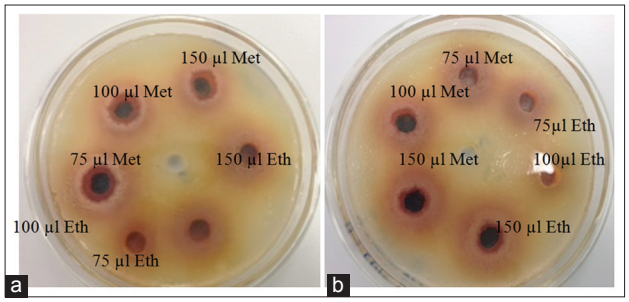

Figure 3: Inhibition zone of bacterial growth using various volumes of date palm leaves 70% ethanol extracts (a) *Staphylococcus aureus*, (b) *Bacillus subtilis*. (Eth denotes ethanol; Met denotes methanol)

Table 2: Antibacterial activity of date palm leaves (Inhibitory zone in mm)

Bacteria/volumes	70% ethanol			Methanol			Amoxicillin
	75 µl	100 µl	150 µl	75 µl	100 µl	150 µl	50 µl
B. subtilis	9	10	10	12	13	13.5	40
E. coli	ND	ND	ND	ND	ND	ND	35
P. aeruginosa	ND	ND	ND	ND	ND	ND	16
S. aureus	12	11	13	12	13.5	15	17

ND: Not definite, *E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, P. aeruginosa: Pseudomonas aeruginosa*

Table 3: MIC

Bacteria	70% ethanol			Methanol		
	100	250	400	100	250	400
B. subtilis	x		✓	x	✓	✓
P. aeruginosa	x	x	x	x	x	x
E. coli	x	x	x	x	x	x
S. aureus	✓	✓	✓	✓	✓	✓

MIC: Minimum inhibitory concentration. ✓: Growth, x: Inhibited.

which was also similar to the studies done by Al-Diahan and Bhat, 2012. And also, they recorded the inhibition zone for aqueous leaf extract to be 10.5 mm which was also similar to our inhibitory zone for *S. aureus*, i.e., 9-11 mm. The results of this study give comparatively small inhibitory zone in *B. subtilius*, i.e., 13.5 where Perveen, *et al*., 2012 found the methanol extract to give an inhibition zone of 21.3 mm. Therefore, our palm leaf extracts showed inhibitory effect only for Gram-positive bacteria.

Date palm leaves' extracts were further examined to find MIC for *B. subtilis* and *S. aureus*. For *B. subtilis* MIC was found as 250 mg/ml for ethanol and methanol and for *S. aureus* MIC was 100 mg/ml for water, ethanol, and methanol.

The leaf extract of the common plant *Bixa orellana* recorded the MIC against the *S. aureus* and the *P. aeruginosa* to be 18.88 and 75.54 mg/ml, respectively. The MIC observed by the leaf extract of mango species *Mangifera indica* against the *S. aureus* was around 75 mg/ml (Doughari and Manzara, 2008). Relatively, the palm leaf extracts used were in high concentrations for the MIC against bacterial strains.

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